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Factors associated with subsequent nontuberculous mycobacterial lung disease in patients with a single sputum isolate on initial examination

M.-R. Lee, MD[1,2], C.-Y. Yang, MD[2], C.-C. Shu, MD[3], C.-K. Lin, MD[1,2], Y.-F. Wen, MD[1,2], S.-W. Lee, MD[4], J.-C. Ko, MD, PhD[1,2], J.-Y. Wang, MD, PhD[1], L.-N. Lee, MD, PhD[2,5] and C.-J. Yu, MD, PhD[2]

1) *Department of Internal Medicine, National Taiwan University Hospital, Hsin-Chu Branch, Hsin-Chu,* 2) *Department of Internal Medicine,* 3) *Department of Traumatology, National Taiwan University Hospital, Taipei,* 4) *Department of Internal Medicine, Taoyuan General Hospital, Taoyuan City and* 5) *Department of Laboratory Medicine, National Taiwan University Hospital, Taipei, Taiwan*

Abstract

Very few studies have focused on the outcome and management of patients with a single sputum isolate of nontuberculous mycobacterium (NTM) on initial examination. Patients with a single isolate of *Mycobacterium avium* complex (MAC), *M. chelonae-abscessus*, *M. kansasii*, or *M. fortuitum* from at least three sputum samples collected within 1 month were retrospectively identified. Those with follow-up sputum samples within 1 year were included in the analysis. Among the 202 patients included, *M. fortuitum* (n = 71, 35.1%) and MAC (n = 70, 34.7%) were the most common NTM species isolated, followed by *M. chelonae-abscessus* (n = 40, 19.8%) and *M. kansasii* (n = 21, 10.4%). The mean clinical follow-up period was 26.2 months. Forty-four patients (21.8%) had subsequent positive cultures of the same NTM species, while eight (4.0%) had bronchiectasis and developed NTM lung disease (NTM-LD). Neither patients without bronchiectasis nor those with *M. fortuitum* subsequently developed NTM lung disease. Among bronchiectatic patients with NTM other than *M. fortuitum*, age $\leq$65 years (p 0.006, OR 32.13), malignancy (p 0.048, OR 14.35), and initial radiographic score >2 (p 0.027, OR 20.06) were associated with subsequent NTM-LD. In all of the NTM patients, bronchiectasis (p <0.001, OR 5.46) and age $\leq$65 years (p 0.002, OR 3.29) were significantly associated with subsequent positive NTM culture. In patients with a single isolation of NTM from respiratory specimens, the presence of bronchiectasis and younger age indicates higher risk of subsequent culture-positivity and NTM-LD. Single isolation of *M. fortuitum* is of little clinical significance. Other patients with NTM, younger age, and more severe radiographic pulmonary lesion also warrant further attention.

Keywords: Atypical mycobacterial infections, bronchiectasis, nontuberculous mycobacterium, respiratory infections, single sputum isolate
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Corresponding author: J.-Y. Wang, Department of Internal Medicine, National Taiwan University Hospital, #7, Chung-Shan South Rd., Taipei 100, Taiwan
E-mail: jywang@ntu.edu.tw

Introduction

Nontuberculous mycobacterium (NTM) lung disease (LD) has been an emerging disease worldwide over the past decade [1]. The difficulty in its management lies in making a definite diagnosis, because the isolation of NTM can mean either colonization or true disease [2–4]. In most circumstances, the microbiologic criteria require positive culture results from two separate expectorated sputum samples [4]. There are, however, instances in the clinical setting of a single sputum positivity for NTM among multiple sputum samples. Single isolation of NTM in respiratory specimens can be attributed to contamination with environmental NTM and is often considered clinically insignificant [5]. Yet, there are still concerns that single

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NTM isolation from sputum samples can be an early sign of NTM-LD [5].

The 2007 American Thoracic Society (ATS)/Infectious Diseases Society of American (IDSA) statement suggests repeating the sputum smear for acid-fast bacilli and mycobacterial cultures if the initial sputum samples results are nondiagnostic [4]. It is also suggested that patients suspected of having NTM-LD but who do not meet the diagnostic criteria be followed-up until the diagnosis is firmly established or excluded [4]. While repeating excessive sputum examinations is often time and labor consuming, as well as expensive, the optimal duration of follow-up is also undetermined. Although diagnostic procedures such as bronchoscopy are integrated in the ATS/IDSA criteria, it is generally not considered due to its invasiveness and potential risks [4]. Clinical parameters and predictors of NTM-LD are therefore needed to help guide the management of patients with single isolation of NTM from sputum samples. Unfortunately, clinical studies on this topic are limited [5].

This study aimed to characterize patients with a single isolation of NTM within multiple sputum samples and determine clinical factors predictive of NTM-LD in this group of patients.

Materials and methods

Ethics statement

The Institutional Review Board of National Taiwan University Hospital approved the study (NTUH REC: 201309019RIN). The need for informed consent was waived because the retrospective design did not add any risk to the participants.

Subjects

This study was conducted at the National Taiwan University Hospital, a 2900-bed tertiary care center in northern Taiwan. The mycobacterial laboratory registry database covering the period of January 2007 to April 2012 was reviewed to identify patients giving at least three expectorated sputum samples within 1 month but with only a single isolate of NTM species, which included *Mycobacterium avium* complex (MAC), *M. kansasii*, *M. chelonae-abscessus*, and *M. fortuitum*. The selected patients should also have at least one follow-up expectorated sputum sample within the following year. Patients with active pulmonary tuberculosis, recent active tuberculosis (within 6 months after complete treatment), or prior isolation of NTM from respiratory specimens were excluded.

The clinical significance of NTM isolates and the adequacy of treatment were judged according to the guidelines established by the ATS [4]. Accordingly, patients were considered as having NTM-LD if they fulfilled the following criteria: (a) at least two sputum or one bronchial washing/brushing sample, or one lung tissue culture positive for the same NTM species; (b) presence of respiratory symptoms; (c) chest radiography or computed tomography demonstrating new patch(es) of consolidation, exudative, nodular infiltrates, cavitary lesions, or multi-focal bronchiectasis; and (d) exclusion of other pulmonary causes [4,6].

All respiratory specimens sent for mycobacterial culture were processed as previously described [7]. Briefly, NaOH-citrate-*N*-acetyl-L-cysteine was added to each specimen in an equal volume and allowed to settle at room temperature for 15 minutes. After centrifugation, the precipitate was re-suspended in 1.5 mL phosphate-buffered saline (pH 7.4). Culture was performed by inoculating 0.5 mL of sediment onto a Middlebrook 7H11 selective agar with antimicrobials (Remel, Inc., Lexena, KS, USA) and by using the fluorometric BACTEC technique (BACTEC MGIT 960 system; Becton-Dickinson Diagnostic Instrument Systems, Sparks, MD, USA) as previously described [7,8].

Smears for acid-fast bacilli of the processed specimens were stained with auramine-rhodamine fluorochrome and examined by standard procedures [7]. Fluorochrome stain-positive smears were confirmed by the Kinyoun stain method [7]. Mycobacterial species were identified by biochemical testing [9]. Quality-control assessment of National Taiwan University Hospital mycobacterial laboratory was periodically performed by the National Reference Laboratory of the Centers for Disease Control of Taiwan [10].

Data collection

A standardized case record form was used to collect the demographic and clinical data, including age, sex, diabetes mellitus, cirrhosis, malignancy, chronic obstructive pulmonary disease, asthma, prior history of pulmonary tuberculosis, radiographic findings, and the course of anti-NTM treatment.

The number of sputum samples and interval between initial and follow-up examination, as well as the period of clinical follow-up, were recorded. The microbiology follow-up period was defined as the interval between the first and last dates of sputum culture in the same patient. The clinical follow-up period was defined as the interval between the time of first sputum examination and the time of last hospital visit by the same patient.

Chest radiography and/or chest computed tomography findings, including bronchiectasis, cavitation, consolidation, and nodules/masses, were recorded. The definition of bronchiectasis on images was based on previous criteria [11–13]. Radiographic severity was also judged according to previous studies [14,15]. Briefly, each lung was divided into three areas. Each area was rated on a four-point scale of 0–3 for extent of

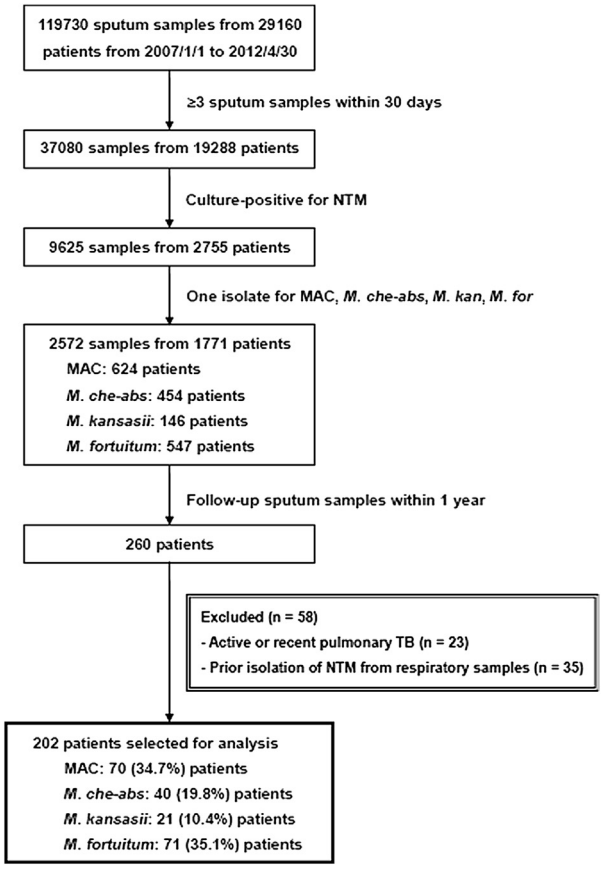

FIG. 1. The patient selection process. MAC, *Mycobacterium avium* complex; *M. che-abs*, *Mycobacterium chelonae-abscessus*; NTM, non-tuberculous mycobacteria; TB, tuberculosis.

infiltration, with a maximum score of 18. The chest images were initially interpreted by two experienced chest specialists. In cases of discrepancy, the final recording was made by consensus.

Primary outcome was set as subsequent development of NTM-LD. Secondary outcome was subsequent culture positivity for the same NTM species.

Statistical analysis

Proportions or means were used to describe the demographic, clinical, and radiographic characteristics. Inter-group differences were analyzed using independent-sampled *t* test for continuous variables and χ^2 test for categorical variables. Logistic regression analysis was used to identify factors associated with subsequent NTM-LD and culture positivity for NTM on follow-up. In the stepwise variable selection procedure, all of the potential predictors were included. Significance levels for entry and stay were set at 0.15. A two-sided $p < 0.05$ was considered significant. All analyses were performed using the SPSS v13.0 (SPSS, Inc., Chicago, IL, USA).

Results

In the patient selection process (Fig. 1), 260 persons with a single isolate of NTM from sputum samples and at least one follow-up sputum sample in the following year were identified covering the study period of January 2007 to April 2012. Twenty-three patients with active or recent pulmonary tuberculosis were excluded, as well as 35 with prior isolate of NTM from respiratory specimens and the single isolate of NTM in the course of follow-up or treatment. A total of 202 patients were enrolled for analysis.

Based on the demographic data of the 202 patients (Table 1), their median age was 70 years, and there was male preponderance (59%). Only one was a child (a 13-year-old boy) and none was found to have cystic fibrosis. *M. fortuitum* (n = 71, 35.1%) and MAC (n = 70, 34.7%) were the most common NTM species isolated, followed by *M. chelonae-abscessus* (n = 40, 19.8%). The mean microbiology follow-up period was 12.0 months, but was significantly shorter in the *M. fortuitum* group (8.1 months). The mean clinical follow-up period was 26.2 months, with no significant differences among groups. The most common radiographic findings were bronchiectasis (n = 70, 35%), followed by consolidation (n = 35, 17%). A majority of the patients had a radiographic score $\leq$2 (n = 127, 63%).

The initial culture-positive sputum samples were smear-negative in all except one patient, an 80-year-old woman with a single isolate of *M. chelonae-abscessus*. She was deemed as not having NTM pulmonary disease, as judged by ATS criteria on initial examination and during follow-up. Forty-four (21.8%) patients had subsequent positive cultures, including 19 MAC, 12 *M. fortuitum*, 8 *M. chelonae-abscessus*, and 5 *M. kansasii* patients. All of them had positive follow-up cultures within the first year. Among the 44 patients, the initial isolates were identical to the follow-up isolates, while six of them also had mixed cultures at follow-up. The co-cultured NTM isolates other than the initial NTM, however, were neglected either because of the insignificance of the species (e.g. *M. gordonae*) or minority in overall cultures.

Of the 202 patients, 137 had no follow-up sputum samples beyond 1 year (microbiology follow-up period $\leq$1 year) and the other 65 did (microbiology follow-up period >1 year). Of the latter, 43 were negative for follow-up cultures within and beyond the first year. The remaining 22 patients had positive follow-up cultures within and beyond the first year.

Of the 202 patients, eight developed NTM-LD on follow-up, including six MAC, one *M. chelonae-abscessus*, and one *M. kansasii*. The median interval between diagnosis of NTM-LD and initial positive culture date was 4.5 months (range 1–23 months). Based on their characteristics, the eight (4.0%)

TABLE 1. Clinical characteristics of patients with a single isolation of NTM ($n = 202$)

Characteristic	Total ($n = 202$)	MAC ($n = 70$)	*M. che-abs* ($n = 40$)	*M. kansasii* ($n = 21$)	*M. fortuitum* ($n = 71$)
Age	70 [13–99]	71 [32–93]	70 [48–89]	71 [45–99]	68 [13–93]
Male	120 (59%)	47 (67.1%)	21 (53%)	16 (76%)	36 (51%)
Number of sputum samples within 1 year	7.8 ± 3.5	8 ± 3.54	7.8 ± 3.8	7.4 ± 3.2	7.6 ± 3.3
Microbiology follow-up period, months	12.0	14.9	12.7	14.2	8.1
Clinical follow-up period, months	26.2	28.8	26.9	24.8	23.7
Presence of subsequent positive culture	44 (22%)	19 (27%)	8 (20%)	5 (24%)	12 (17%)
Diagnosed as NTM pulmonary disease	8 (4%)	6 (9%)	1 (3%)	1 (5%)	0
Age	57 [48–70]	57 [49–64]	48	70	NA
Male	5 (63%)	4 (67%)	0	1 (100%)	NA
Months to 2nd positive cultures	2 [1–11]	1.5 [1–11]	2	6	NA
Months to diagnosis of NTM-LD (from single isolate of NTM)	4.5 [1–23]	2.5 [1–23]	7	6	NA
Microbiology follow-up months	22.5 [1–39]	17.5 [1–31]	39	16	NA
Clinical follow-up months	36 [12–60]	36 [12–60]	39	19	NA
Underlying disease					
Old pulmonary tuberculosis	35 (17%)	15 (21%)	5 (13%)	5 (24%)	10 (14%)
Chronic obstructive pulmonary disease	35 (17%)	16 (23%)	4 (10%)	5 (24%)	10 (14%)
Malignancy	62 (31%)	24 (34%)	11 (28%)	5 (24%)	22 (31%)
Diabetes	36 (18%)	12 (17%)	9 (23%)	2 (10%)	13 (18%)
CKD (serum creatinine >2 mg/dL)	17 (8%)	7 (10%)	5 (13%)	0	5 (7%)
Radiographic findings					
Bronchiectasis	70 (35%)	28 (40%)	10 (25%)	10 (48%)	22 (31%)
Cavitary	6 (8%)	1 (1%)	1 (2%)	2 (10%)	2 (3%)
Nodules	10 (14%)	2 (3%)	1 (3%)	3 (14%)	4 (6%)
Consolidation	35 (17%)	11 (16%)	13 (33%)	3 (14%)	8 (11%)
Radiographic score ≤2	127 (63%)	40 (57%)	24 (60%)	12 (57%)	51 (72%)

CKD, chronic kidney disease; LD, lung disease; MAC, *Mycobacterium avium* complex; *M. che-abs*, *Mycobacterium chelonae-abscessus*; NA, not available; NTM, nontuberculous mycobacteria.
Data are mean ± SD, median [range], or number (%).

patients who subsequently developed NTM-LD were mainly middle-aged adults. All except one (case 2) had subsequent positive culture immediately on the next follow-up. The eight NTM-LD patients were also judged by their primary care physician to have NTM-LD. Among them, three had image deterioration, two had symptoms progression, and three had both image deterioration and symptoms progression.

None of the 71 patients in the *M. fortuitum* group had another positive culture and subsequent NTM-LD, whereas eight (6.1%) in the remaining 131 patients did (p 0.034, by χ^2 test). Of the 131 patients, none of the 83 patients without bronchiectasis developed NTM-LD during follow-up, compared to eight (16.7%) of the remaining 48 patients who did (p 0.001, by χ^2 test). Thus, "*M. fortuitum*" was a significant predictor of no subsequent positive culture. Together with "no bronchiectasis," it was also a significant predictor of nondevelopment of NTM-LD.

Because neither nonbronchiectatic patients nor those with *M. fortuitum* subsequently developed NTM-LD, only 48 bronchiectatic patients with NTM species other than *M. fortuitum* were included in the data in fitting the logistic regression model for the primary outcome of developing NTM-LD. Age ≤65 years (p 0.006, OR 32.13), presence of malignancy (p 0.048, OR 14.35), and initial radiographic score >2 (p 0.027, OR 20.06) were associated with subsequent NTM-LD (Table 2).

Of the 202 patients with a single isolate of NTM, bronchiectasis (p <0.001, OR 5.46) and age ≤65 years (p 0.002, OR 3.29) were significant predictors of the secondary outcome (subsequent positive cultures for NTM) (Table 2).

Discussion

In this study, 4.0% of patients with single sputum positivity for NTM developed NTM-LD in a mean follow-up time of 12 months. Bronchiectasis was a strong predictor of subsequent positive culture and NTM-LD among patients with single

TABLE 2. Factors associated with subsequent NTM lung disease in bronchiectatic patients ($n = 48$) and subsequent culture positivity for NTM in all patients ($n = 202$) with a single isolate of *Mycobacterium avium* complex, *M. chelonae-abscessus*, or *M. kansasii* ($n = 48$), by multivariate logistic regression analysis

Outcome	Variables	Percent(s) of patients developing outcome	p	OR (95% CI)
Subsequent NTM lung disease	Age: ≤65 vs. >65 years	32% vs. 4%	0.006	32.13 (2.72–379.33)
	Malignancy: Present vs. Absent	30% vs. 13%	0.048	14.35 (1.02–201.13)
	Initial radiographic score: >2 vs. ≤2	20% vs. 14%	0.027	20.06 (1.40–286.25)
Subsequent culture-positivity for NTM	Age: ≤65 vs. >65 years	33% vs. 15%	0.002	3.29 (1.56–6.92)
	Bronchiectasis: Present vs. Absent	40% vs. 12%	<0.001	5.46 (2.59–11.49)

NTM, nontuberculous mycobacteria.

sputum positivity for MAC, *M. chelonae-abscessus*, and *M. kansasii*. Younger age, malignancy, and higher radiographic score were also associated with subsequent NTM-LD. Single isolate of *M. fortuitum* from multiple sputum samples was of little clinical significance. The chance of subsequent sputum positivity was low if follow-up sputum samples within 1 year yielded no positive culture for the same NTM species.

In a previous study, only 2% of patients with a single isolate of MAC developed radiologic evidence of disease and progression within 12 months of follow-up [16]. Another study found that 14% of patients with a single sputum culture positive for pathogenic NTM were diagnosed with NTM-LD within a median follow-up period of 16 months [5]. These findings suggest that a single isolation of NTM can be an early sign of lung disease and patients can progress to lung disease in subsequent follow-up [5]. Another possibility is that these patients are under-diagnosed because they produce little sputum or have inadequate skills for sputum collection [4,17]. Although there is a chance of transient environmental exposure or laboratory contamination causing a single NTM isolation, a certain proportion of patients still demonstrate clinical deterioration and are later diagnosed with NTM-LD [5,17]. The results of this study were consistent with previous reports and remind physicians to be aware of possible clinical deterioration and development of disease for patients with initial single sputum isolate for NTM.

Bronchiectasis is considered an important underlying lung disease contributing to NTM-LD [18,19]. Infections with NTM have also been postulated to contribute to the initiation of bronchiectasis [20], especially since the isolation of NTM is a common finding among patients with bronchiectasis, with a frequency reportedly up to 37% [13]. In fact, bronchiectasis itself is even a radiographic characteristic integrated in the ATS diagnostic criteria for NTM-LD [4]. The most common NTM species isolated in patients with bronchiectasis is MAC. The clinical significance of NTM isolation among patients with bronchiectasis remains debatable, with the exception that NTM isolation in cystic fibrosis bronchiectasis often indicates clinical disease [21,22]. It is not surprising that bronchiectasis is the single strongest factor predicting subsequent positive NTM culture and NTM-LD. The present findings also indicate that the colonization of NTM (one single positive culture) is less likely to persist or even progress to lung disease in the absence of structural lung conditions.

The finding that younger age is associated with a higher risk of subsequent NTM-LD and subsequent positive cultures for NTM is interesting. It can be hypothesized that this is partly associated with greater functional impairment among older patients [23]. Younger patients can have better expectoration skills and the sputum samples are more adequate, thus lessening the risk of a false-negative result or contamination [24]. Furthermore, younger age is also a risk for greater decline in lung function among patients with NTM isolates from respiratory specimens. Together with the present study, younger age may be a prognostic factor predictive of faster clinical deterioration among patients with NTM isolates [14,25].

Malignancy has also been proposed as a risk factor for NTM-LD. The association is believed to be secondary to defective cell-mediated immunity [26]. A higher radiographic score is also associated with subsequent NTM-LD, probably reflecting a greater severity of NTM colonization and thus, a higher risk of clinical deterioration [14,27].

Six of the eight patients who developed subsequent NTM-LD are MAC patients with bronchiectasis. This phenomenon may also stem from the belief that MAC patients with nodular-bronchiectatic type produce less or little sputum [17,28]. Initial sputum examinations can be a non-yielder for NTM in this group of patients [28]. As such, a more aggressive approach, like bronchoscopic examination with bronchial washing or even trans-bronchial lung biopsy, may be considered in this patient group if initial sputum results are inconclusive [29].

M. fortuitum is considered less virulent. Its clinical significance in respiratory specimens is not well established, although it is considered to cause colonization or transient infection in patients with underlying lung diseases [30]. Among patients with two or more sputum specimens that are culture positive for *M. fortuitum*, very few demonstrate clinical deterioration and require antibiotic treatment [30]. Few studies also discuss the clinical significance of a single isolation of *M. fortuitum*. In the present study, although patients with a single isolation of *M. fortuitum* can have subsequent positive cultures, none were further diagnosed as having *M. fortuitum*-LD. The clinical significance of a single isolation of *M. fortuitum* may therefore be trivial.

The optimal follow-up duration for patients with a single isolation of NTM remains unclear. In a previous study, 97% of the first two positive cultures grew from the initial three sputum specimens [4]. In the study by Koh *et al.*, 14% of patients with a single isolation of NTM had subsequent NTM isolation and were diagnosed with NTM-LD [5]. The median duration of the first sputum culture to the second sputum culture was 30 months (interquartile range 15–46 months) [5]. In the 65 patients with follow-up sputum samples with an interval for more than 1 year of follow-up in the present study, one-third had subsequent positive sputum and all within 1 year. Nonetheless, 43 patients remained negative for NTM. These findings suggest that the chance of sputum positivity decreases significantly after 1 year. Lengthy follow-up with sputum examinations may not be cost effective.

This study has its limitations, mainly due to its retrospective design. Follow-up duration, imaging modalities, and timing of sputum acid-fast smear and mycobacterial culture were not standardized. Therefore, the incidence of NTM-LD is likely to be underestimated and it is difficult to know at which moment patients began to have NTM-LD. We also did not perform detailed species identification on MAC and *M. chelonae-abscessus*. Nonetheless, this study intended to provide a simple guideline to help clinicians cope with a frequently encountered scenario. Detailed species results from molecular techniques, while providing more information, are not readily available for many physicians. Also, we did not include all NTM species in our patient selection. MAC, *M. chelonae-abscessus*, *M. kansasii*, and *M. fortuitum*, however, are the most common NTM species with clinical significance isolated in respiratory specimens in Taiwan [2–4]. *M. xenopi* was not included due to its rarity in our institute [2,3].

In conclusion, 4% of patients with a single isolation of NTM from respiratory specimens subsequently develop NTM-LD. Young patients with bronchiectasis, underlying malignancy, and high radiographic score warrant further attention. Single isolation of *M. fortuitum* may be of little clinical significance. The risks of subsequent lung disease decrease significantly if the next follow-up sputum examinations yield negative findings within 1 year.

Transparency declaration

The authors declare that they have no conflicts of interest.

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